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EXECUTIVE SUMMARY
TO

CARCINOGENS AND MUTAGENS IN AMBIENT AIR PARTICULATE MATTER: SOURCES AND TRENDS IN CONTRA COSTA COUNTY

Contract No. ARB A1-162-32

Final Report

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ABSTRACT

Many mutagens and carcinogens are known to be present in urban community air. Extensive chemical and biological characterization of these atmospheric pollutants is essential if accurate risk assessments are to be made and effective control strategies developed. This report describes progress in three areas of this complex environmental problem: 1. the development of more sensitive methods for measuring aerosol mutagens, 2. the identification of sources of mutagens and 3. the analysis of trends in mutagen and polycyclic aromatic hydrocarbon (PAH) levels in particulate organic matter (POM).

- A highly sensitive version of the Ames Salmonella test, called the microsuspension test, was applied to measure the mutagenic activity in organic extracts of community aerosols. Application of the microsuspension Ames test made possible high resolution diurnal studies of mutagenicity in small air samples of only 2 hours duration. Diurnal variations in mutagenic density (revertants/m^3) of more than a factor of 10 were observed and these variations were highly correlated with fine fraction lead (Pb) in a pilot field study. The test can be applied in future studies where sample mass is a limiting factor.

- The origins of mutagens in POM were investigated further by sampling in Contra Costa County during six seasonal pollution episodes, each of 36 hours duration, in 1982-1984. Samples were collected at four locations (Richmond, Martinez, Concord, Pittsburg) and analyzed for mutagenic activity in the Ames test, for PAH, oxyanions (NO_3^-, $SO_4^=$), pollutant gases (CO, NO, NO_2, O_3, SO_2) and elemental source tracers (including Pb, Br, Ni, Fe and K). Diurnal, geographic and seasonal comparisons were made. Statistical techniques, including principal component (factor) analysis, were used to explore relationships between aerosol mutagens, PAH and source tracers. The results confirmed earlier observations and provided some new insights into the sources of aerosol mutagens.

    (i) Several lines of evidence indicate that some mutagenic aerosols are primary automotive pollutants emitted directly into the atmosphere.

a. In this present and previous Contra Costa studies, mutagenic density and PAH were significantly positively correlated with fine fraction (< 2.5 μmd_a) Pb and/or Br, both derived primarily from motor vehicles.

b. Chemical analysis by other investigators has identified mutagens (various PAH and nitroarenes) in on-road vehicle particulate emissions, as well as other combustion source particulate matter.

c. Studies of upwind-downwind freeway data in Los Angeles by Statewide Air Pollution Research Center (SAPRC) scientists have demonstrated an incremental burden of direct mutagens in aerosol attributable to freeway traffic. The amount was comparable to the area wide background mutagen density.

(ii) Many results suggest that some mutagens behave as secondary aerosols. The hypothesis that some mutagenic aerosols are formed in the atmosphere is supported by the following evidence:

a. During pollutation episodes in Contra Costa County, mutagens were positively correlated with NO_3^-, assumed to be a secondary aerosol tracer. The association of mutagenicity with NO_3^- occurred area-wide.

b. SAPRC scientists observed that ratios of mutagen densities (rev/m^3) to CO were generally higher at Riverside, California, a downwind receptor site, than at El Monte, an intermediate receptor site in the Los Angeles basin. Since CO is an unreactive combustion emission, the mutagen density/CO ratio takes into account variations in emissions and atmospheric dispersion. Higher ratios at Riverside suggest atmospheric mutagen formation during aerosol transport from Los Angeles.

c. The ratios of mutagenic densities to Pb which we have measured in Contra Costa County in this and a previous study were highest

during summer episodes when the prevailing atmospheric conditions (i.e. hot, stagnant) favored chemical transformations. Since Pb, like CO is an unreactive emission, the mutagenic density/Pb ratio should take into account variations in automotive emission profiles and dispersion. Thus the high ratios during episodes in August 1981 and September 1983 may reflect atmospheric mutagen formation.

d. Smog chamber studies have demonstrated the formation of nitro-PAH mutagens. Mutagenicity of some nitro-PAH's exceed the mutagenicity of the parent PAH by several orders of magnitude in laboratory analysis. Some of these highly mutagenic nitro-PAH's are known to be primary pollutants emitted by various combustion sources. However chamber studies have also shown that irradiation of mixtures of atmospheric hydrocarbons, nitric acid (HNO_3) and reactive gases (NO_2, O_3) can lead to mutagen formation. Thus some hydrocarbons may be converted to secondary mutagenic products under simulated atmospheric conditions.

e. Measurements in a nitroreductase mutant indicate the likely presence of nitroarene mutagens. Less than 10% of the total mutagenicity in ambient air samples is due to identified PAH. Thus most of the mutagenicity remains to be explained in chemical terms. A substantial proportion of this excess mutagenicity may be due to highly mutagenic nitroarenes and derivatives, which are not only ubiquitious primary pollutants but may also be derived from secondary atmospheric transformations. We infer that such compounds were probably major contributors to the mutagenicity of Contra Costa aerosols from the fact that mutagenic activities of aerosol extracts were two to three times lower in a Salmonella strain (TA98NR) deficient in an enzyme required for some mono-nitroarene activation, than in the standard tester strain (TA98).

f. Finally, measurement artifacts confound the secondary mutagen hypothesis. The positive correlations of mutagenic density with NO_3^-, and the demonstration that mutagenic organic compounds can be formed under simulated atmospheric conditions support the hypothesis of secondary formation of mutagenic aerosols in the atmosphere. The association between mutagens and NO_3^- can be influenced by HNO_3 artifacts produced by sampling on glass fiber filters. There are two concerns. Gas phase HNO_3 can bind to glass fiber and artificially increase apparent particulate NO_3^- concentrations. More importantly, gas phase HNO_3 may catalyze chemical tranformations of PAH to produce highly mutagenic nitroaromatic compounds during sample collection on glass fiber. The significance of these potential artifacts cannot be assessed accurately at present.

(iii) For the first time in Contra Costa County, industrial contributions to mutagenic aerosols were suggested by significant positive correlations between mutagenic density and S (both fine fraction S and SO_2) at Richmond and Martinez. Sulfur oxides are major air pollutants in the vicinity of large oil refineries and chemical plants in Contra Costa County. The major industrial sources of SO_2 are refineries in Richmond (Chevron), Martinez (Shell, Tosco) and Benicia (Exxon) and a chemical plant in Rodeo (Union).

- Routine collection and analysis of 4 month seasonal composite filter samples was carried out in Contra Costa County between 1979-1984. The three periods were Nov.-Feb., March-June and July-Oct. These periods approximate the three meteorological seasons in the area.

This monitoring effort demonstrated that levels of most aerosol pollutants including mutagens and PAH, were highest in the winter (Nov.-Feb.).

A prime goal of the monitoring was to detect any time trends which may have occurred. Monitoring did indeed reveal a positive trend in the concentration of mutagenic aerosols, despite decreasing or constant levels of the other pollutants

measured. The annual average increased from 5 revertants/m^3 in 1979-80 to 19 revertants/m^3 in 1983-84. A three to four-fold increase in mutagenic density (from 8 revertants/m^3 to 27 revertants/m^3) was observed over the five winter seasons. Values in the spring increased from 2 to 18 revertants/m^3 while summertime values increased by more than a factor of two from 5 to 13 revertants/m^3. Further monitoring is needed to determine the persistence of these trends.

PROJECT SUMMARY

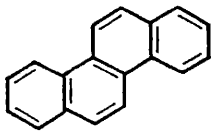
A. Introduction and Statement of the Problem

A variety of chemical mutagens and carcinogens are known to be present in particulate matter in urban community air (1-9). Recent epidemiological conclusions place the number of cancer deaths in the U.S. due to environmental pollution (air, water and soil) at 8,000 annually (7). It has been estimated that outdoor community air pollution alone may be the cause of about 15% of the present lung cancer cases among non-smoking residents in Contra Costa County (8), neglecting contributions from indoor air pollution. Thus it may be argued that exposure to mutagens and carcinogens in outdoor community air remains a significant public health concern which warrants continued monitoring and investigation.

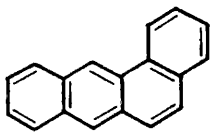
Carcinogenic chemicals found in air particulate matter include certain polycyclic aromatic hydrocarbons (PAH), such as benzo(a)pyrene (BaP) and benzo(k)fluoranthene (9). Structures of these and other PAH are shown in Figure I. However, these chemicals constitute only a small fraction of the total carcinogenic potential. Organic extracts of ambient particulate organic matter (POM) are significantly more carcinogenic and mutagenic than expected on the basis of the amounts of the measured chemicals present (10,11). The excess mutagenicity, as defined by the Ames test (12), reflects a significant gap in our knowledge. The gap may be due to our inability until recently to measure small concentrations of carcinogenic and highly mutagenic nitroarenes (e.g. 1-nitropyrene) (13) which are present in diesel exhaust particulates (14) and urban air (15,16,17). These compounds probably account for a significant portion of the observed mutagenicity of urban air particulate matter.

However, the measurement of mutagenicity in a given geographical area and even the chemical identification of the mutagens present are of limited public health value unless the major sources of mutagenicity can be identified and controlled. With few exceptions, the sources of mutagens and carcinogens in air particulates are unknown. In Contra Costa County, for example, PAH

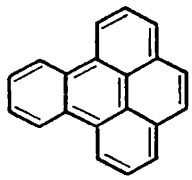
Figure 1



Chrysene



Benz(a)anthracene



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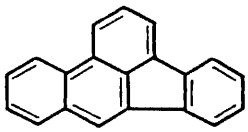
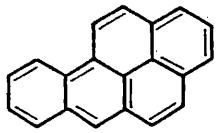
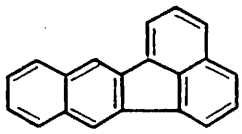


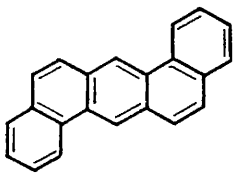
Benzo(b)fluoranthene



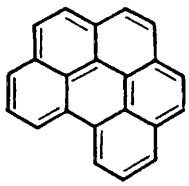
Benzo(a)pyrene



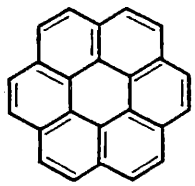
Benzo(k)fluoranthene



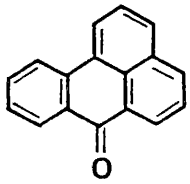
Dibenz(a,h)anthracene



Benzo(ghi)perylene



Coronene



7-H-benz(d,e)anthracen-7-one

FIGURE I STRUCTURE AND NOMENCLATURE OF 10 POM's

appeared to be derived principally from vehicular emissions but these compounds account for less than ten percent of measured mutagenicity (6). Until recently the principal sources of mutagens remained obscure.

Concern about sources of mutagen led to a previous study of mutagens in Contra Costa County carried out by the Air and Industrial Hygiene Laboratory (AIHL) under contract to the California Air Resources Board (CARB) (18). The AIHL-CARB project provided the background to the present investigation. The previous study measured the mutagenic activity of ambient aerosol extracts and quantitated a number of organic compounds which contribute to the mutagenicity. Air sampling was carried out during three seasonal pollution episodes in 1981-1982. Solvent extracts of POM were analyzed for mutagenic activity in the Ames Salmonella test and for polycyclic aromatic hydrocarbons (PAH) by high pressure liquid chromatography (HPLC) with fluorescence detection. Pb, Ni, Fe, NO_3^-, $SO_4^=$, O_3, CO, NO, NO_2, and SO_2 were also measured. Diurnal variations in mutagenicity and chemical pollutant concentrations were compared. Mutagenicity was found to be consistently and strongly associated with lead-containing fine particles (< 2.5 μmd_a) emitted primarily by gasoline powered vehicles without catalysts. During a winter inversion, possible contributions to PAH from residential wood combustion were also noted.

The previous study also revealed positive correlations between mutagenicity and nitrates during a summer pollution episode. We speculated earlier that this correlation could reflect chemical formation of nitro PAH compounds in the atmosphere and suggested that in some respects, the formation of mutagenic aerosols was similar to the formation of photochemical oxidant; the effects of atmospheric dispersion and transformation both need to be considered. Since this suggestion could have policy implications for control of oxides of nitrogen, further investigation was required.

B. Project Objectives

The present study was designed to followup these earlier leads by addressing three topics which are critical to a further understanding of mutagens and carcinogens in community air: 1) the validation of a more sensitive method

for measuring aerosol mutagens, 2) the identification of the sources and the possible routes of atmospheric chemical formation of aerosol mutagens and 3) the investigation of seasonal variations and the recent trends in airborne mutagen and PAH concentrations in Contra Costa County between 1979 and 1984.

C. Experimental Approach

The research carried out under this and the previous CARB-contract (18), employed a similar experimental approach and methods. The abbreviations of air pollutants and methods used for their collection and analysis are listed in Tables 1 and 2.

1. Application of the Salmonella Microsuspension Procedure to the Measurement of Mutagenicity in Air Particulate Matter

A simple and highly sensitive version of the Salmonella liquid incubation assay (19) was validated using pure chemical mutagens and then applied to the measurement of air particulate mutagenicity. These applications included diurnal studies carried out at several locations. In one experiment, selected criteria pollutants (Pb, NO_2, O_3, SO_2) were sampled concurrently, to provide information about sources of mutagens.

2. Intensive Sampling for Mutagen and PAH Source Identification

A second focus of the present effort was on source identification. Six pollution periods were sampled intensively to investigate sources of mutagens and PAH. The sampling and analytical plan for source identification is shown in Table 3. Separate "day" (0600-1800) and "night" (1800-0600) sampling was carried out over 36 hour episodes. Episodes were stable meteorological periods, when concentrations of pollutants were relatively high. Samples were collected at four locations (Richmond, Martinez, Concord and Pittsburg) in the northern industrialized portion of Contra Costa County (Figure 2). The northern section of the county contains heavy industry including five major petroleum refineries and many chemical

TABLE 1

ACRONYMS FOR AIR POLLUTANT VARIABLES USED IN THE ANALYSIS AND INTERPRETATION OF CONTRA COSTA DATA

TSP	Total Suspended Particulate Mass	$\mu g/m^3$
$SO_4^=$	Sulfate Mass	$\mu g/m^3$
NO_3^-	Nitrate Mass	$\mu g/m^3$
ORG	Benzene Soluble Organics	$\mu g/m^3$
BAP	Benzo(a)pyrene	ng/m^3
BKF	Benzo(k)fluoranthene	ng/m^3
BGP	Benzo(ghi)perylene	ng/m^3
COR	Coronene	ng/m^3
BZO	Benzanthrone (all isomers)	ng/m^3
PBF	Fine fraction Lead	ng/m^3
BRF	Fine fraction Bromine	ng/m^3
FEF	Fine fraction Iron	ng/m^3
SIF	Fine fraction Silica	ng/m^3
KF	Fine fraction Potassium	ng/m^3
ZNF	Fine fraction Zinc	ng/m^3
SF	Fine fraction Sulfur	ng/m^3
CLF	Fine fraction Chlorine	ng/m^3
M398PS9	Revertants per m^3 in TA98+S9	rev/m^3
M398MS9	Revertants per m^3 in TA98-S9	rev/m^3
M398NRM	Revertants per m^3 in TA98NR-S9	rev/m^3
NR/98M3	Revertants per m^3 in TA98NR/TA98	-
ORG98PS9	Revertants per ORG in TA98 + S9	rev/μg
ORG98MS9	Revertants per ORG in TA89-S9	rev/μg
O_3	Ozone	pphm
CO	Carbon Monoxide	ppm
NO	Nitrogen oxide	pphm
NO_2	Nitrogen dioxide	pphm
SO_2	Sulfur dioxide	pphm

TABLE 2

METHODS USED FOR COLLECTION AND ANALYSIS OF PARTICULATE AND GASEOUS AIR POLLUTANTS

Pollutant	Collection (Medium)	Analysis
TSP	Hi-vol (glass fiber)	Gravimetric
$SO_4^=$	Hi-vol (glass fiber)	Turbidimetric
NO_3^-	Hi-vol (glass fiber)	Colorimetric
ORG	Hi-vol (glass fiber)	Benzene extraction
PAH	Hi-vol (glass fiber)	HPLC - fluorescence
Mutagens	Hi-vol (glass fiber)	Standard Ames test
	Dichotomous (Teflon)	Microsuspension Ames test
Trace elements	Dichotomous (Teflon)	X-ray fluorescence
	Hi-Vol (glass fiber)*	" "
O_3	Dasibi, Model 1003-AH	Ultraviolet absorption
NO, NO_2	Thermal-electron, Model 14D	Chemiluminescence
CO	Bendix, Model 8301-5CA	Infrared absorption
SO_2	Thermal-electron, Model 43	Fluorescence

*Pb only.

TABLE 3

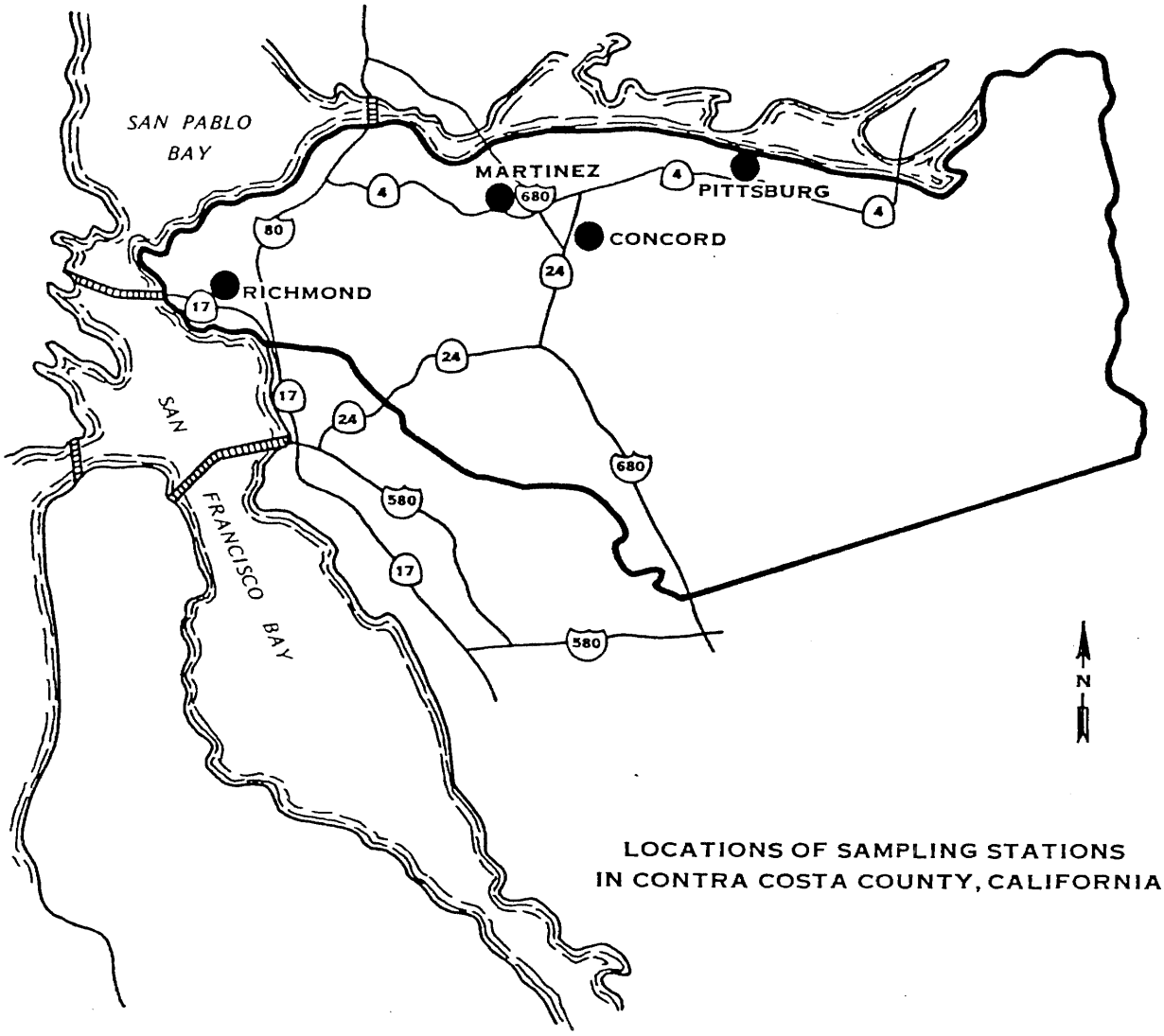
SAMPLING AND ANALYTICAL PLAN FOR MUTAGEN SOURCE IDENTIFICATION

Day-Night Collection: 6 a.m. - 6 p.m.
6 p.m. - 6 a.m.

Collect Particulates on:

	Analytical Method	Agency Performing Analysis
Hi-vol #1 (glass fiber)		
Mass	Gravimetric	AIHL
Sulfate	Turbidimetric	AIHL
Nitrate	Colorimetric	AIHL
Lead	X-ray Fluorescence	AIHL
Organics	Benzene Extraction	AIHL
Hi-vol #2 (glass fiber) Refrigerate immediately		
Mutagenicity	Ames	AIHL
PAH	HPLC	AIHL
Dichotomous Samplers (membrane or Teflon filter)		
Multielemental analysis	Energy Dispersive X-ray Fluorescence	DHS-LBL
Collect Gas Data		
NO_x	Chemiluminescence Photometry	BAAQMD
CO	Non-Dispersive Infrared Absorption	BAAQMD
SO_2	Fluorescence Photometry	BAAQMD
O_3	Ultraviolet Absoprtion	BAAQMD
Collect Meteorological Data		
Wind direction Wind speed		BAAQMD

FIGURE 2



LOCATIONS OF SAMPLING STATIONS
IN CONTRA COSTA COUNTY, CALIFORNIA

plants. Three of the stations (Richmond, Concord, Pittsburg) are part of the Bay Area Air Quality Management District (BAAQMD) network. Martinez was a temporary site, adjacent to a petrochemical refinery.

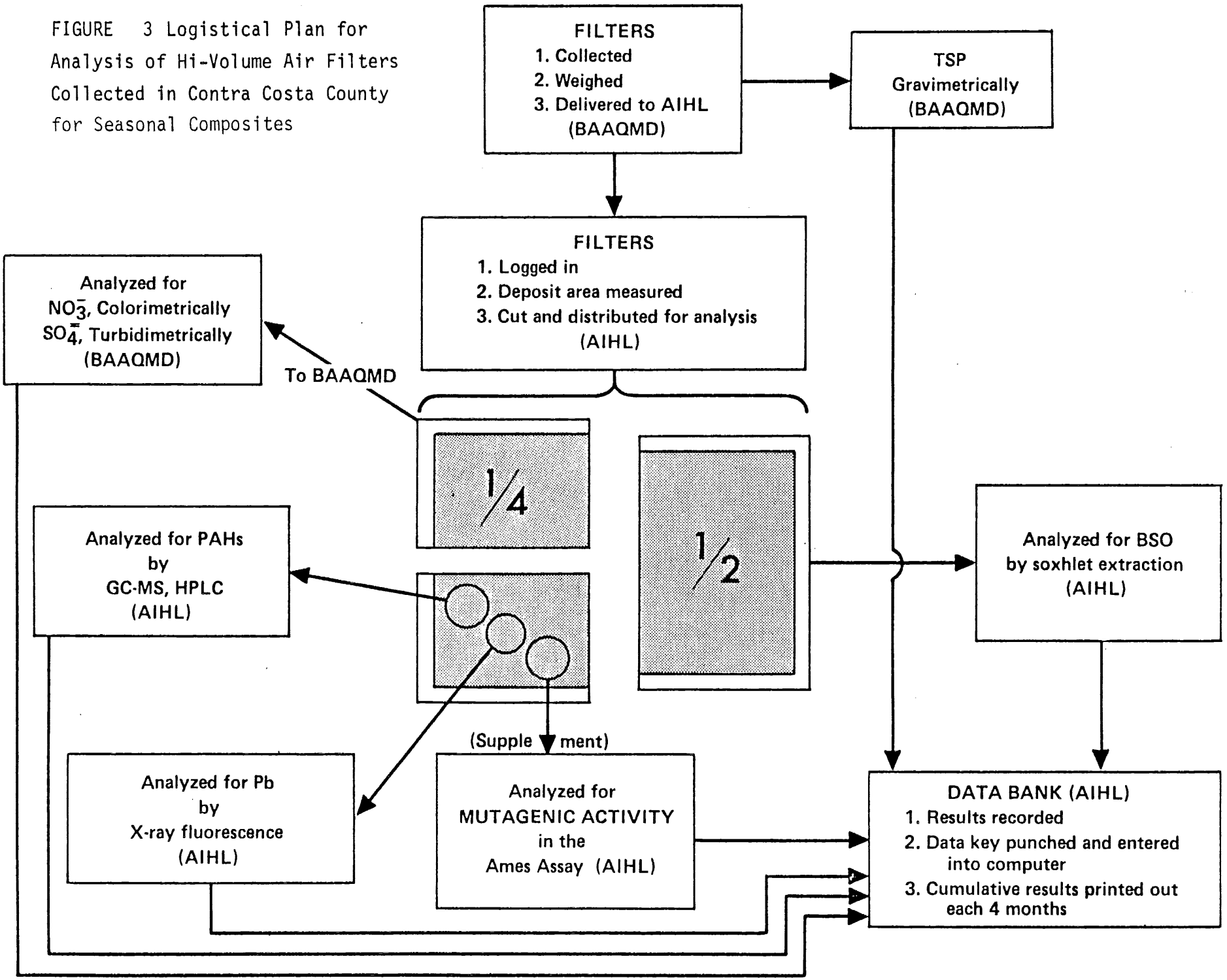
Each location had samplers to collect air particulate matter for analysis of mutagenicity, PAH, trace metals (including Pb, Ni, K, Si), NO_3^-, $SO_4^=$ and total mass. Gaseous pollutants (CO, SO_2, NO, NO_2, O_3) were also measured. At Martinez, wind speed and direction were obtained. Chemical and mutagenicity data were combined using simple and complex statistical methods in an attempt to identify sources of mutagens and selected PAH.

3. Collection and Analysis of Seasonal Composites

To determine seasonal variations and trends, samples were collected at the same three permanent stations of the BAAQMD network (Concord, Pittsburg and Richmond) used for intensive sampling. Hi-vol filter samples were collected every sixth day at each station for routine monitoring purposes and were analyzed for total suspended particulate (TSP), $SO_4^=$, NO_3^-, organics and Pb. A portion of each filter was composited for PAH and mutagenicity testing. Each station was composited separately. The logistical plan for analysis of hi-vol filters collected for seasonal composites is shown in Figure 3. Filters from each of the three stations were composited over four-month intervals (July-October, November-February, March-June), to give composite samples for analysis. These periods approximate the three meteorological seasons in the San Francisco Bay air basin and also correspond with those used in our previous studies in Contra Costa County (18).

Samples collected during the period July 1982-October 1984 were composited and analyzed for PAH and mutagenic activity. When combined with results of previous studies, these provide a continuous data base of the concentrations of specific PAH and mutagenic activity in Contra Costa air particulate material collected over five years, since November 1979. Results of PAH and mutagenicity measurements in composite samples were also compared with TSP, NO_3^-, $SO_4^=$, Pb and total organics on a season-by-season basis.

FIGURE 3 Logistical Plan for Analysis of Hi-Volume Air Filters Collected in Contra Costa County for Seasonal Composites



D. Summary of Findings

Efforts to validate and apply a highly sensitive version of the Ames test to air samples (Chapter II) yielded the following findings:

1. The 10 fold increased sensitivity of the "microsuspension" Ames test made possible high resolution diurnal studies of mutagenicity in small samples of only 2 hours duration (Figure 4).

2. Diurnal variations in mutagenic density (rev/m^3) of more than a factor of 10 were observed.

3. Diurnal variations in mutagenic density were highly correlated with fine fraction Pb, in a pilot field study.

4. The test can be applied in future studies where sample mass is a limiting factor.

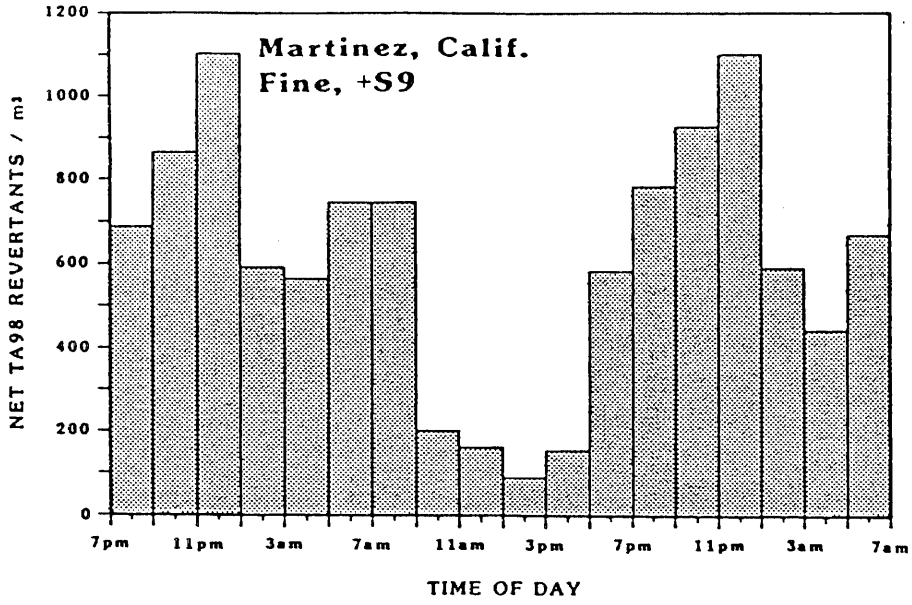
Intensive episode sampling and analysis for source identification (Chapter III) confirmed earlier observations and provided now new insights into sources of aerosol mutagens.

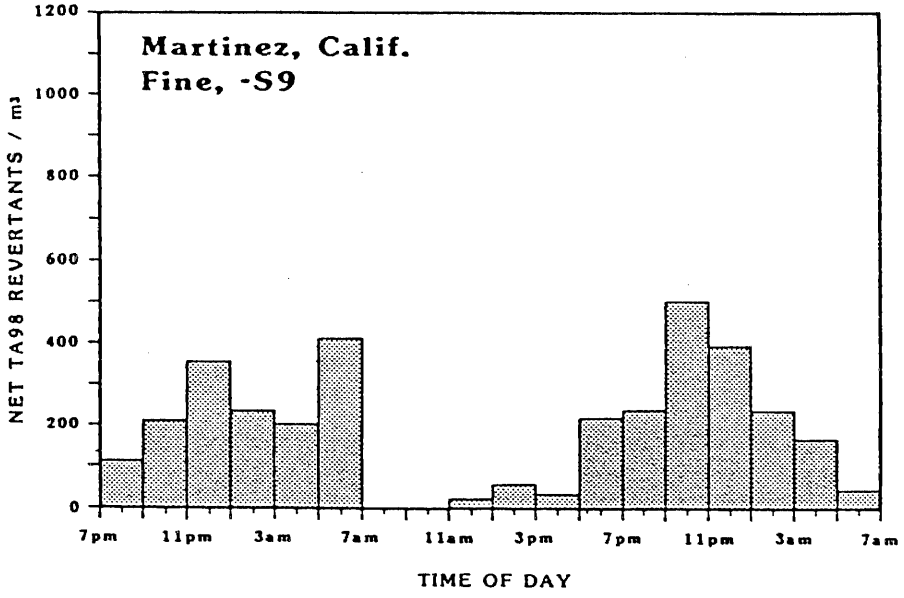
1. Several lines of evidence indicate that some mutagenic aerosols are primary automotive pollutants emitted directly into the atmosphere.

    a. In this and earlier Contra Costa studies mutagens (and PAH) were significantly correlated with fine fraction Pb and Br, indicating contributions from primary automotive emissions (Table 4).

    b. Chemical analysis by other investigators has identified mutagens (various PAH and nitroarenes) in on-road vehicle particulate emissions (20) as well as other combustion source particulate matter (21).

FIGURE 4 Diurnal variation of mutagenicity of fine airborne particles collected in Martinez, California and measured in the microsuspension procudure. TA98 with S9 (a); TA98 without S9 (b); TA98 NR without S9 (c).





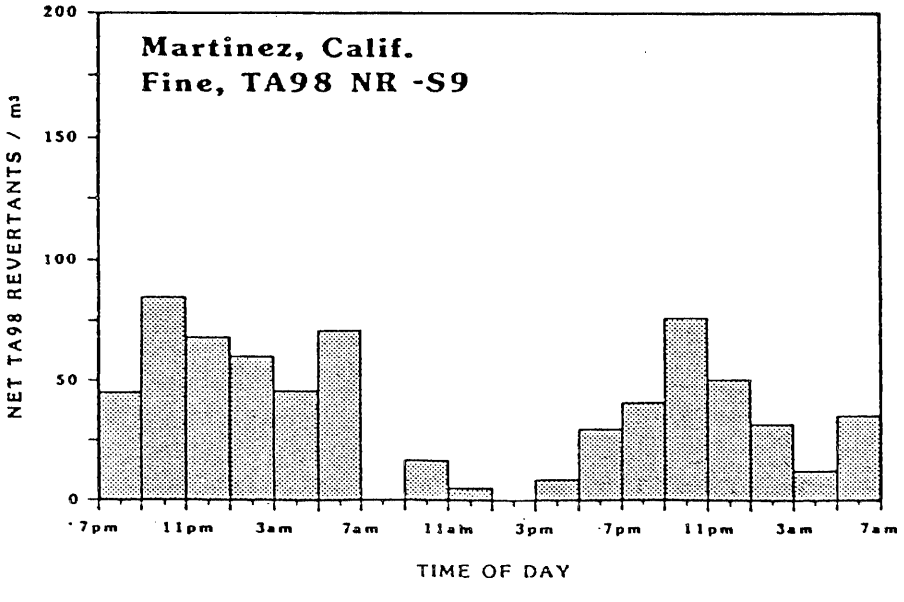


TABLE 4

CORRELATIONS BETWEEN MUTAGENIC DENSITY (rev/m^3), SELECTED PAH AND AIR POLLUTANTS: COMBINED EPISODE DATA, 1982-1984

	TA98+S9	TA98-S9	BAP	COR	BZO
TA98+S9	1.00	0.94**	0.45**	0.54**	0.60**
TA98-S9	0.94**	1.00	0.49**	0.44**	0.59**
BAP	0.45**	0.49**	1.00	0.38**	0.92**
COR	0.54**	0.44**	0.37**	1.00	0.56**
BZO	0.60**	0.59**	0.92**	0.56**	1.00
PBF	0.28*	0.25*	0.08	0.71**	0.19
BRF	0.40**	0.38**	0.30**	0.74**	0.44**
KF	0.26*	0.22	0.15	0.58**	0.08
ZNF	0.12	0.12	-0.01	0.27*	0.26
FEF	0.09	0.13	-0.07	0.28*	-0.05
SIF	-0.12	0.01	-0.07	0.13	-0.09
CLF	-0.27*	-0.22	0.01	-0.15	-0.05
NIF	-0.10	-0.07	-0.12	-0.20	-0.12
SF	0.22	0.23*	0.28*	0.03	0.36**
NO_3^-	0.49**	0.51**	0.32**	0.15	0.47**
CO	0.54**	0.51**	0.23	0.71**	0.36**
NO	0.51**	0.45**	0.34**	0.49**	0.44**
NO_2	0.32**	0.31**	0.11	0.61**	0.18
O_3	-0.27*	-0.35**	-0.41**	-0.13	-0.43**
SO_2	0.04	0.05	-0.02	-0.27*	-0.02

*Significant at the $p \leq 0.05$ level.

**Significant at the $p \leq 0.01$ level.

c. Studies of upwind-downwind freeway data in Los Angeles by Sweetman et al (22) have demonstrated an incremental burden of direct mutagens in aerosol attributable to freeway traffic which was comparable to the area wide background mutagen density.

2. Many results suggest that some mutagens behaved as secondary aerosols. The hypothesis that some mutagenic aerosols are formed in the atmosphere is strengthened by the following evidence:

a. During pollution episodes in Contra Costa County, mutagens were positively correlated with NO_3^-, assumed to be a secondary aerosol tracer. The association of mutagenicity with NO_3^- occurred area-wide.

b. Pitts and co-workers (23) observed that ratios of mutagen densities (rev/m^3) to CO were generally higher at Riverside, a receptor site, than at El Monte, an intermediate receptor location in the Los Angeles basin. Since CO is an unreactive combustion emission, the mutagen density/CO ratio takes into account variations in emissions and atmospheric dispersion. Higher ratios at Riverside suggest atmospheric mutagen formation during aerosol transport from Los Angeles.

c. The ratios of mutagenic densities to Pb which we have measured in Contra Costa County in this and a previous study (18) were highest during summer episodes when the prevailing atmospheric conditions (i.e. hot, dry, stagnant) favored chemical transformations. Since Pb, like CO, is an unreactive emission, the mutagenic density/Pb ratio should also take into account variations in (automotive) emission profiles and dispersion. Thus the high ratios during episodes in August 1981 (18) and September 1983 may reflect atmospheric mutagen formation.

d. Smog chamber studies have demonstrated the formation of nitro-PAH and other mutagens. Mutagenicity of some nitro-PAH's exceed

the mutagenicity of the parent PAH by several orders of magnitude in laboratory analysis. Some of these highly mutagenic nitro-PAH's are known to be primary pollutants emitted by various combustion sources. However chamber studies (24,25) have also shown that irradiation of mixtures of atmospheric hydrocarbons, nitric acid (HNO_3) and reactive gases (NO_2, O_3) can lead to mutagen formation. Thus some some hydrocarbons may be converted to secondary mutagenic products under simulated atmospheric conditions.

e. Measurements in a nitroreductase mutant indicate the likeley presence of nitroorganic mutagens. Less than 10% of the total mutagenicity in ambient air samples is due to identified PAH. Thus most of the mutagenicity remains to be explained in chemical terms. A substantial proportion of this excess mutagenicity may be due to highly mutagenic nitroarenes, which are not only ubiquitious primary pollutants but may also be derived from secondary atmospheric transformations. We infer that nitroarenes were probably major contributors to the mutagenicity of Contra Costa aerosols from the fact that mutagenic activities of aerosol extracts were two to three times lower in a Salmonella strain (TA98NR) deficient in an enzyme for some mononitroarene activation, than in the standard tester strain (TA98). With respect to mutagenicity of community air collected in other cities this finding is not unique. For example, air particulate samples from Los Angeles (23) and Detroit (26) also showed markedly reduced mutagenic activities in nitroreductase deficient strains.

f. Finally, measurement artifacts confound the secondary mutagen hypothesis. The positive correlations of mutagenic density with NO_3^- and the demonstration that mutagenic organic compounds can be formed under simulated atmospheric conditions support the hypothesis of secondary formation of mutagenic aerosols in the atmosphere. However interpretation is complicated by measurement artifacts in nitrates and nitro-aromatic compounds. The association between mutagens and NO_3^- could be influenced by HNO_3 artifacts

produced by sampling on glass fiber filters. There are two concerns. Gas phase HNO_3 can bind to glass fiber and artificially increase apparent particulate NO_3^- concentrations (27). More importantly, gas phase HNO_3 may catalyze chemical transformations of PAH to produce highly mutagenic nitroaromatic compounds during sample collection on glass fiber (13). The significance of these potential artifacts can not be assessed accurately at present.

3. For the first time, industrial contributions to mutagenic aerosols were also suggested by significant positive correlations between mutagenic density and S (both fine fraction S and SO_2) at Richmond and Martinez. These sulfur oxides are major air pollutants in the vicinity of large oil refineries and chemical plants concentrated in Contra Costa County. The major industrial sources are refineries in Richmond (Chevron), Martinez (Shell, Tosco) and Benicia (Exxon) and a chemical plant in Rodeo (Union) (28).

Routine collection and analysis of seasonal composite filters in Contra Costa County between 1979-1984 (Chapter IV) revealed both seasonal variations and trends.

1. Concentrations of mutagens, PAH and the standard air pollutants (TSP, Pb, NO_3^-, $SO_4^=$) were highest during the winter (Nov.-Feb.) season. PAH exhibited the greatest seasonal changes, 3-10 fold. High wintertime PAH concentrations could reflect contributions from residential wood combustion.

2. A positive trend in concentrations of mutagenic aerosols ($\pm$S9) was found between 1979 and 1984. For example, a nearly four-fold increase in the annual average mutagenic density (+S9) from 5 to 19 rev/m^3 was observed over the five years of monitoring (Figure 5).

3. The positive trend in mutagenicity was in contrast to the fairly constant (annual average) levels of PAH and the decreasing levels of the standard pollutants. The decrease in Pb was most apparent. For example over

Figure 5

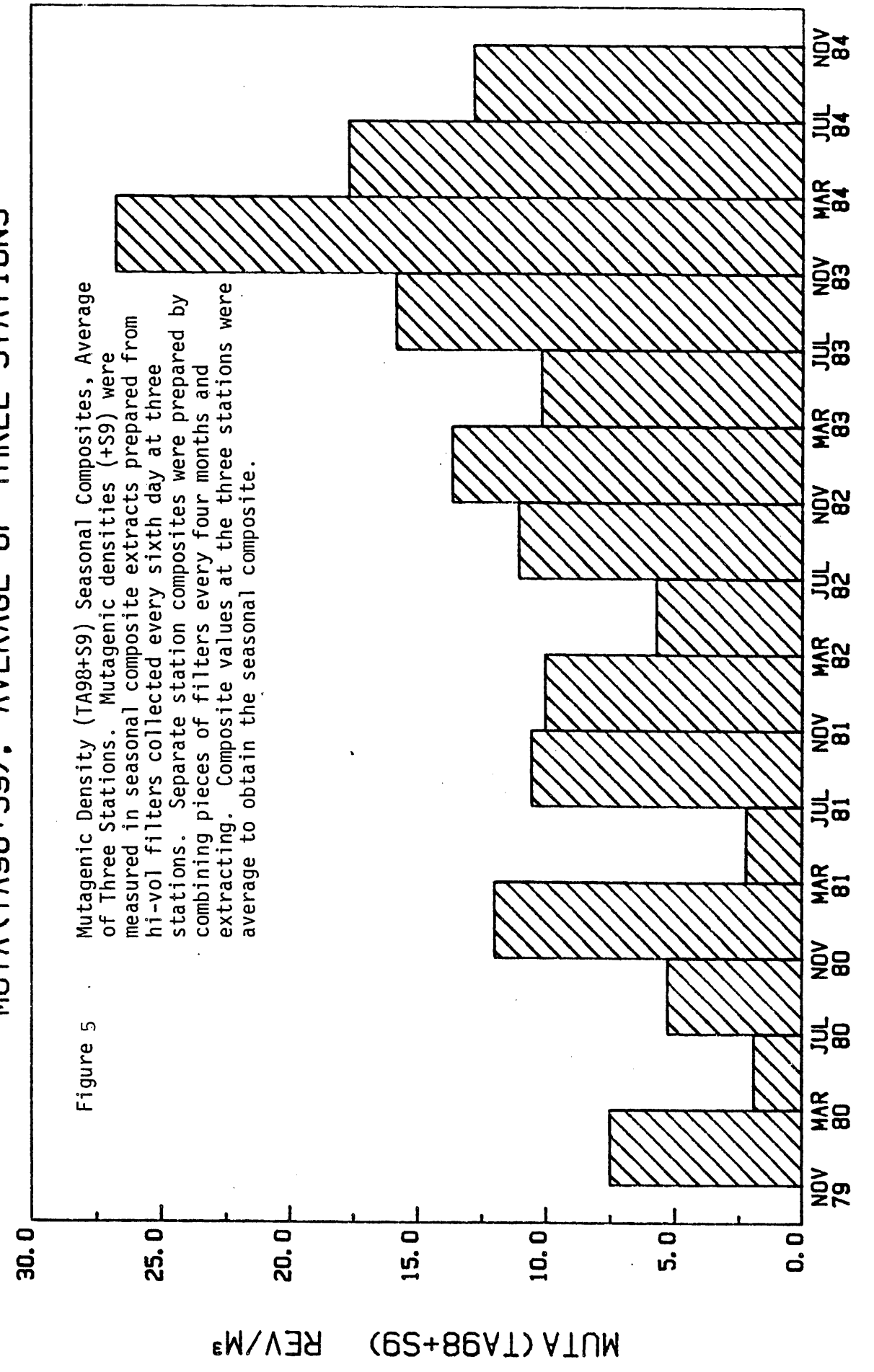


Figure 5 Mutagenic Density (TA98+S9) Seasonal Composites, Average of Three Stations. Mutagenic densities (+S9) were measured in seasonal composite extracts prepared from hi-vol filters collected every sixth day at three stations. Separate station composites were prepared by combining pieces of filters every four months and extracting. Composite values at the three stations were average to obtain the seasonal composite.

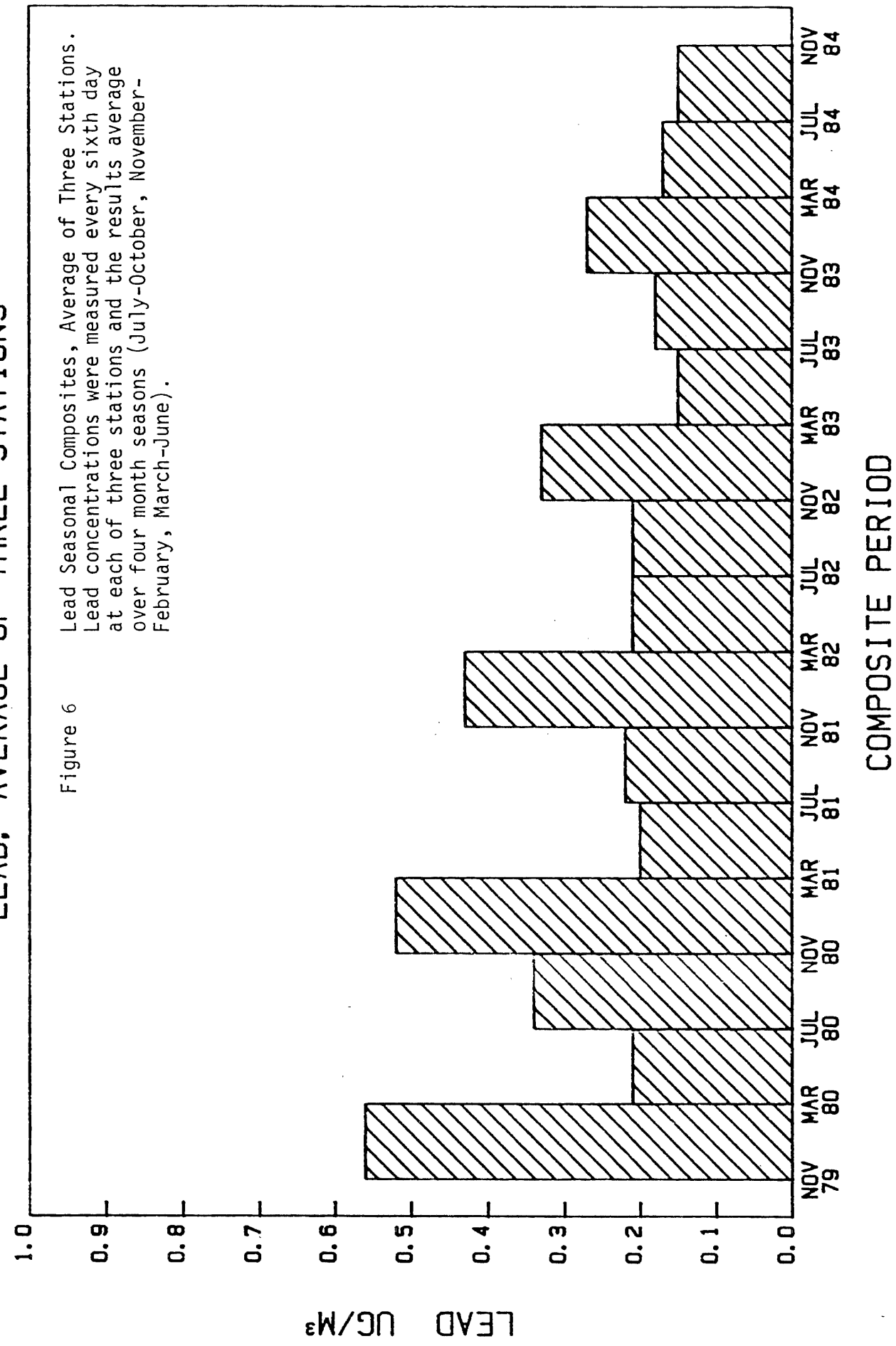


Figure 6 Lead Seasonal Composites, Average of Three Stations. Lead concentrations were measured every sixth day at each of three stations and the results average over four month seasons (July-October, November-February, March-June).

the five winter seasons (1979-1984) Pb decreased from 0.57 ± 0.13 ng/m^3 to 0.27 ± 0.03 ng/m^3. The Pb gasoline phase-out program in the Bay Area, or different meteorological factors for the sampling seasons may be responsible (Figure 6).

E. Recommendations for Future Research

The partial answers derived from the present research effort also generated additional questions for possible future research.

Investigation of sources has lead to the suggestion that mutagens may be formed atmospherically, during normal aging of community aerosols. Before endorsing this suggestion further, several measurement questions must be addressed. As noted above, the apparent association between mutagens and NO_3^- could be influenced by HNO_3 artifacts produced by sampling on glass fiber filters. Gas phase HNO_3 can bind to glass fiber and artificially increase apparent particulate NO_3^- concentrations. Appel and co-workers (27) have recently compared artifact NO_3^- formation on different filter media. Laboratory and atmospheric sampling studies were performed to evaluate glass fiber and Teflon filters for their abilities to form artifact particulate nitrate with HNO_3. At nitric acid dosages representative of those in the atmosphere, glass fiber filters retained >94% of the HNO_3 and Teflon <2% of HNO_3.

Gas phase HNO_3 may also catalyze chemical transformations of PAH to produce highly mutagenic nitroaromatic compounds. These transformations can occur both in the atmosphere and on filters during sample collection. Pitts et al (13) first showed the formation of directly mutagenic nitroderivatives from PAH coated on glass fiber filters and exposed to flows of air containing NO_2 and traces of nitric acid. Extending this research, Pitts and co-workers (23) have more recently studied sampling artifacts utilizing two filter types (glass fiber and Teflon-impregnated glass fiber). The ratios of mutagen densities for POM simultaneously collected on glass fiber and Teflon-impregnated glass fiber varied by more than a factor of ten. The greatest differences occurred during periods of elevated O_3 concentrations, suggesting that under such conditions there is an artifact effect associated with particulate collection (probably) on glass fiber

filters. Ambient concentrations of HNO_3 and other reactive gases (NO_x, O_3) in Contra Costa County are not as high as in El Monte and Riverside, where these artifacts were studied. Nevertheless, direct evaluation of possible HNO_3-glass fiber effects in Contra Costa air samples should be done. Experiments are recommended to compare mutagenicity and NO_3^- values in aerosols collected on glass-fiber and Teflon-impregnated glass fiber filters in samplers equipped with or without HNO_3 denuders.

A further recommendation concerns industrial emissions. We have observed for the first time in Contra Costa County significant positive correlations between mutagenicity and the petrochemical tracer S at Richmond and Martinez. Petrochemical and other chemical sources may therefore contribute to mutagenic emissions. Follow-up research on stationary source emissions should be done. This research should provide sampling methods for both volatile and aerosol mutagens; at Richmond and Martinez mutagenicity was positively correlated with gaseous SO_2, as well as fine S aerosols.

A final recommendation is to maintain and expand the monitoring network for mutagens and PAH, in light of the increasing trends in mutagenicity observed in recent years. To verify the trend analysis, routine monitoring should continue in Contra Costa County and be extended to include other high pollution locales in the Bay Area (e.g. southern Santa Clara County) and adjacent air basins (e.g. Sacramento-San Joaquin Valley, Chico to Bakersfield). Existing air sampling networks would be used. Because samples are routinely collected at sites in these networks, and Ames and PAH testing are routinely carried out in AIHL, the cost would be minimal.

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